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14. ABSTRACT

Targeting HER2 is not as straightforward as it was originally predicted since many breast tumors express multiple HER receptors and co-express one or more HER ligands. This cross-talk network negatively impacts the response to the currently used HER2-targeted agents, highlighting the urgent need for a novel anti-HER2 molecule(s) presenting a combination strategy. Activation of the HER2 receptor is attained by several means: 1) receptor overexpression leads to homo-dimerization; 2) EGF induces EGFR-HER2 hetero-dimerization by binding to EGFR and inducing HER2 activation via crosstalk; and 3) Heregulin (HRG) induces HER3-HER2 hetero-dimerization by activating HER2 via its binding to the HER3 and HER4 receptors, in turn inducing receptor crosstalk and activation of a signaling cascade. In short, our preliminary study reveals that the specific disruption of an essential activating sequence existing on HER2 ECD domain III is capable of disabling the HER2 homo- and hetero-dimerization, thus blocking activation of HER2-driven oncogenic signaling and generating a "dominant-negative" Peptides or compounds with specificity for this functional site should add a previously unrecognized molecular approach to our therapeutic arsenal for the management of HER2-overexpressing carcinomas.

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Table of Contents

INTRODUCTION

Amplification of the HER2 gene leading to overexpression of the HER2 receptor occurs in ~30% of breast carcinomas and correlates with more aggressive breast cancer disease (1). The association of HER2 overexpression with enhanced malignant phenotypes of breast cancer cells, including those with metastatic potential and resistance to chemo- and endocrine-therapies, provides a plausible interpretation for the poor clinical outcome of breast cancer patients with HER2-overexpressing tumors (2-6). HER2 is the only HER family receptor member that does not bind known ligands. In cancer cells, however, this "ligandless" receptor tyrosine kinase can be activated by two ways: by overexpression, which has been proposed to cause a degree of spontaneous dimerization and activation in the absence of ligand, or by heterodimerization with another (ligand-stimulated) HER receptor (7-11). Antibodies directed against the HER2 ECD and tyrosine kinase inhibitors that target its cytoplasmic kinase domain are in clinical use or at advanced developmental stages (12-16). Unfortunately, many breast tumors express multiple HER receptors at different levels and co-express one or more HER ligands, and this negatively impacts on the response to currently used HER2-targeted agents - highlighting an urgent need for novel anti-HER2 molecules presenting a "combination strategy" (17).

The increasing number of newly discovered anti-cancer agents comes as a result of understanding the ultimate mechanisms underlying malignant transformation and metastatic potential, rather than as a result of random screening of molecules. HER2 represents a successful example of this molecular approach. Experimental studies demonstrating that high levels of HER2 transform cultured cells as well as clinical studies showing poorer long-term survival rates for patients whose tumors overexpress HER2 implied that HER2 should be suitable as a therapeutic target (1-6). While various approaches have been taken to target this molecule, the most prominent strategy for the treatment of HER2-overexpressing carcinomas has involved antibody-targeting of the ECD of HER2 (6, 18-23).

To date, two therapeutic strategies have met with some success for suppressing aberrant HER signaling in human cancer: Antibody targeting of the extracellular region of the receptor and small-molecule inhibition of the cytoplasmic tyrosine kinase domain. Two anti-HER2 antibodies (*i.e.*, trastuzumab and pertuzumab) bind the untethered, dimerization-competent state of HER2 (17). The HER2-targeted antibody trastuzumab, used for treatment of metastatic breast cancer patients whose tumors overexpress HER2, binds to domain IV of HER2 ECD (40). Therefore, trastuzumab does not prevent heterodimerization and fails to inhibit the growth of tumor displaying low levels of HER2 because HER1/3 ligands can induce the formation of HER2-containing HER1-HER2 and HER3-HER2 heterodimers in the presence of the antibody (17). Pertuzumab, another HER2-targeting antibody, binds HER2 near the centre of the domain II dimerization arm, thereby preventing autocrine or paracrine HER3 ligands from inducing HER2-HER3 heterodimerization and, therefore, having no growth inhibition effects without exogenous ligand stimulation (32, 33).

The crystal structure of the HER1 ECD in complex with the antigen binding fragment of the anti-HER1 monoclonal antibody cetuximab recently revealed that the antibody footprint specifically overlaps that of EGF on domain III (L2) of HER1, thus blocking the interaction of domain I and III and preventing ligand-induced activation of the receptor. (41, 42). Cetuximab, however, binds to the tethered ("closed") state of HER1 though direct ligand competition (41, 42). Crystallographic studies (9, 40) have shown that the HER2 ECD is 'autoactivated' when compared with the ECDs of EGFR (43) and HER3 (44). A key dimerization arm that is buried in unactivated EGFR and ErbB3 is clearly exposed in HER2 even without bound ligand (45). In the case of EGFR, binding of EGF or TGFα to subdomains I and III is required to expose the dimerization arm (46, 47). The HER2 ECD is unique in having direct interactions between HER2 domains I & III could effectively mimic ligand binding. Currently, there is no HER-targeted therapeutics that specifically affects the interaction

between subdomains I and III within the HER2 ECD, which it is the ultimate responsible inducing a constitutively extended and activated homo- (HER2-HER2) and hetero- (HER1-HER2, HER2-HER3) dimerization loop. We therefore hypothesized that blocking these direct interactions between HER2 domains I & III should inactivate HER2 so that it cannot participate in efficient homo- and hetero-dimerization. We predicted that, with this 'autoactivating' interaction blocked, HER2 would fail to provide either a substrate for *trans*-phosphorylation or an active catalyst for transmission of the mitogenic and/or pro-survival signaling in the absence or presence of HER ligands.

BODY

1: Design and expression of ECD deletion derivatives of HER2: HER receptor family members contain four domains within their ECD (45). Domains I and III are α-helix domains that both bind simultaneously to the activating ligand in the case of the EGF receptor (46, 47). By bringing domains I and III close to one another, EGF (or TGFα) exposes a dimerization arm in domain II, and also induces other conformational changes that are important for EGFR dimerization (45, 48). Structures of the HER2 ECD (which has no known ligand) indicate that a direct interaction between subdomains I and III serves to extend the molecule constitutively so that its dimerization arm is always exposed (9, 40, 45). With its constitutively activated configuration, ligandless HER2 is thus thought to heterodimerize with itself or other HER receptor tyrosine kinases (10). With this model in mind, we introduced a series of six deletion mutations into domain III of the HER2 ECD in an attempt to disrupt the autoactivating interaction between domains I and III so disablingHER2 signaling (Fig. 1).

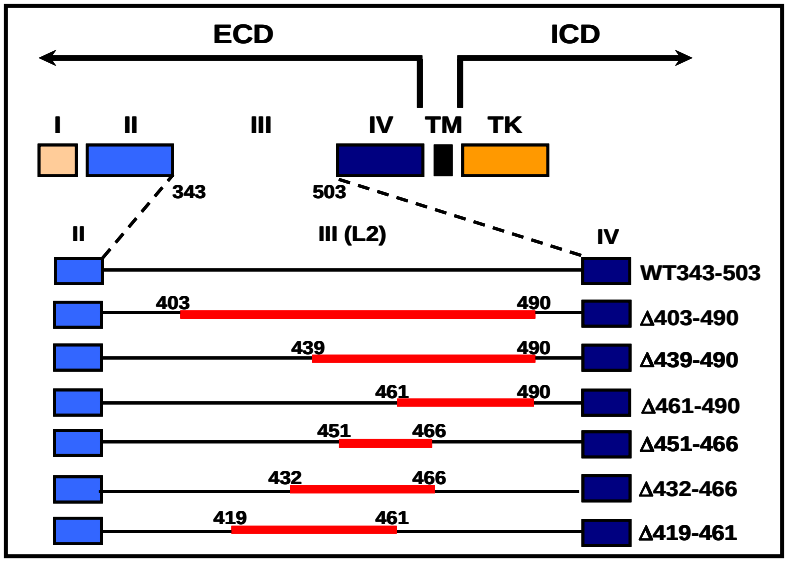

Figure 1: *Schematic representation of the wild-type HER2 receptor and HER2 ECD deletion mutants.* ECD: Extracellular domain; ICD: Intracellular domain; TK: Tyrosine kinase. Domains II and IV are cysteine cluster domains. Domains I and III are putative ligand or dimerization domains. The deletion mutants of the HER2 receptor were generated on the region of domain III between the two cysteine cluster domains, a putative functional site. The sequences deleted are indicated as a red line, and the numbers indicate the exact regions that were deleted for each mutant.

Deletions were made in the context of full-length HER2, and cloned into the pLXSN vector for expression in mammalian cells. To determine the biological effects of these deletions on HER2 function, we have taken advantage of the breast epithelial cell line MCF10A, a non-transformed, near diploid, spontaneously immortalized human mammary epithelial cell line. These cells provide a useful *in vitro* system to analyze the effect of expressing specific genes implicated in breast cancer development in a non-transformed genetic background (49, 50). In addition to expressing very low levels of HER2 and HER3, MCF10A cells express HER1 (EGFR) and are dependent on EGF for optimal cell growth. Additionally, we have used different HER2 overexpressing breast cancer cell lines SK-Br3 and BT474 with different biological phenotypes as described later in the preliminary data section.

2: Deletions in domain III of the HER2 ECD inhibit HER2 activation: When the expression of wild-type and ECD deletion forms of HER2 was assessed by immunoblotting analyses, proteins of the expected sizes were detected with a mouse monoclonal antibody directed against the carboxyl terminal 14 amino acids of HER2

(Figure 3). A striking picture emerged when the activation status of HER2 (pHER2) in the absence of added EGF was monitored with a monoclonal antibody (clone PN2A) that specifically recognizes the phosphorylated tyrosine 1248 residue, which constitutes the main autophosphorylation site of HER2. All six HER2 deletion mutants demonstrated decreased tyrosine kinase activities (less intense pHER2 bands) when compared to the phosphorylation status of the wild-type form of HER2 (Fig 2).

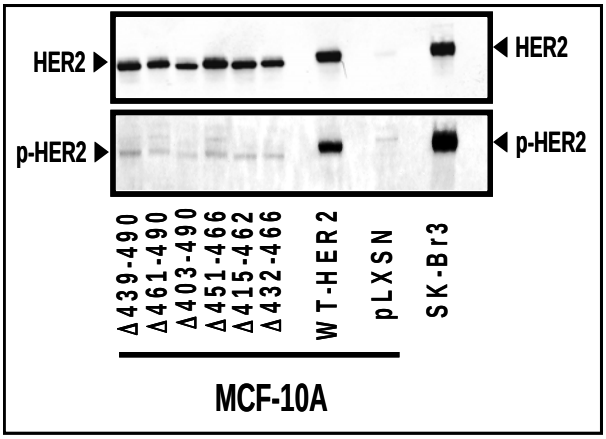

Figure 2: *Effects of HER2 ECD structural deletions on HER2 autophosphorylation.* 20 μg of total protein from 75-80% confluent cultures of SK-Br3 cells (positive control for HER2 overexpression and overactivation), MCF10A cells (negative control for HER2 overexpression and overactivation) and MCF10A cells engineered to stably express the wild-type form of HER2, HER2 ECD deletion mutant derivatives or the empty vector pLXSN, all of which growing in complete medium, were resolved by 3-8% Tris-Acetate NuPAGE and subjected to immunoblotting analyses for HER2 and phospho-HER2 using anti-HER2 and anti-phospho-HER2 monoclonal antibodies.

It is known that HER2 can be phosphorylated as a consequence of HER1 interaction by heterodimerization of the two receptors or by direct phosphorylation of HER2 by activated HER1. Overexpression of HER2 inhibits the down-regulation of HER1, thus resulting in constitutive activation of HER1 in a ligand-independent manner (10, 51-55). Co-expression of HER1 and HER2 exists in many breast carcinomas with the worst prognosis (56). This complex cross-talk between the two wild-type HER receptors is recapitulated in our experimental model because some EGF-independent hyperactivation of HER1 could be detected in wild-type HER2-overexpressing MCF10A cells using a monoclonal antibody that specifically recognizes phosphorylated tyrosine 1173 in HER1 (pHER1, Fig 3A). The constitutive activation of HER1 (pHER1) seen in the wild-type HER2-transduced cell line was abolished by the smallest deletion Δ451-466 in the HER2 ECD (Fig 3A). Deletion of these 15 amino acids also prevented activation phosphorylation of HER2 at Y1248 following stimulation with EGF, but did not affect EGF-induced activation of HER1.Exogenous supplementation with Heregulin-β_1 (HRG-β_1), a growth factor cloned based on its ability to induce tyrosine phosphorylation of HER2 mainly through the formation of HER2/HER3 heterodimeric complexes, also failed to activate Δ6 HER2 (data not shown). To initially explore a "dominant-negative effect¨ following disruption of an autoactivating interaction within the HER2 ECD, we examined steady-state levels of activated HER2 as well as downstream mitogen-activated protein kinase (MAPK)-ERK1/2 and PI-3K/AKT growth/survival signaling cascades in MCF10A/wild-type HER2, MCF10A/Δ6 HER2 and MCF10A/pLXSN matched control cells growing in complete medium.

First, expression of Δ6 HER2 seemed to block the activity of the low levels of endogenous HER2 endogenously expressed in MCF10A cells (Fig 3B, top panel). Second, the activation status of MAPK upon the overexpression of Δ6 HER2 significantly decreased when compared to wild-type HER2-induced hyperactivation of MAPK. Indeed, forced expression of Δ6 HER2slightly decreased the basal MAPK activation observed in the control MCF10A/pLXSN cells (Fig 3B, bottom left panel). This "dominant-negative effect" became more evident when analyzing the steady-state levels of AKT phosphorylated at its activating residue Ser^{473}, which were greatly repressed in MCF10A/Δ6 cells (Fig 3B, bottom right panel). A critical up-stream component of the AKT transduction cascade, HER3 contains six docking sites for PI-3K p85 subunit and therefore couples HER2 very efficiently to the PI-3K/AKT pathway (11, 17, 57). These findings suggest that the Δ6 HER2 profoundly affects the ability of HER2 to cross talk with activated HER1 and impairs ligand-induced *trans*-activation of HER2 by HER3.

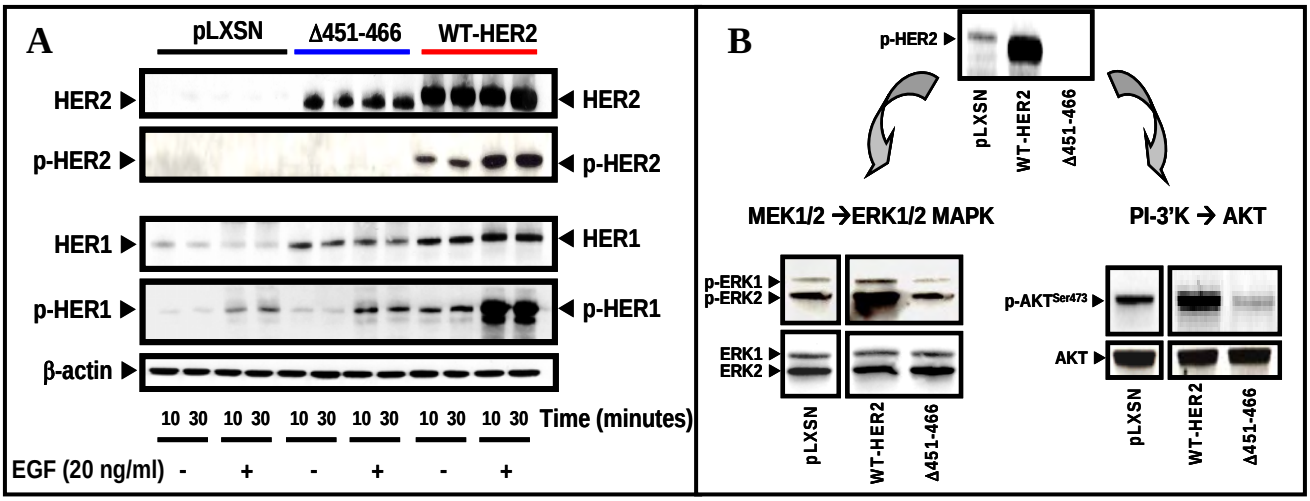

Figure 3: *Effects of the HER2 ECD deletion mutant Δ451-466 (Δ6) on HER2-driven signaling in MCF10A cells.* **(A)** Effects of Δ6 HER2 on the upstream HER1-HER2 cross-talk. After MCF10A/wild-type HER2, MCF10A/HER2 MCF10A/Δ6 HER2 and MCF10A/pLXSN matched control cells were serum starved overnight, EGF at 20ng/ml was added for 10 or 30 minutes. 50 μg of total protein from each experimental condition were loaded per lane. **(B)** Effects of Δ6 HER2 on the downstream effectors ERK1/2 and AKT.

***3: The smallest deletion in sub-domain III of the HER2 ECD (Δ6) blocks HER2-promoted EGF independence*:** We next investigated the biological consequences of Δ6HER2-driven malignancy by evaluating well-characterized HER2-promoted transforming phenotypes in normal breast epithelial cells (49, 50). First, we assessed the effect of the Δ6 HER2 on the ability of HER2 to subvert the EGF-dependence of MCF10A cells (Fig 4A). EGF significantly stimulated MCF10A/pLSXN cells. Conversely, EGF had no significant effect on the growth of MCF10A cells overexpressing HER2. The growth in the absence of EGF were significantly lower for cells expressing Δ6 HER2for control MCF-10A/pLXSN cells (Fig 4A); further suggesting that Δ6 HER2 exerts a "dominant-negative" effect. To determine the effect of the Δ6 HER2 on EGF-promoted cell-cycle progression, cells, were incubated in the presence of EGF for 24 h (Fig 4B). In the absence of EGF stimulation, the response of MCF10A cells to Δ451-466 deletion was characterized by a > 3-fold decrease in the percentage of cells undergoing S-Phase as well as an accumulation of cells in the G_1-phase of cell cycle (Fig 4B, top panels) and Δ6HER2-promoted G_0/G_1 cell cycle blockade. The fraction of cells in S-phase was as low as 6% in EGF-stimulated MCF10A/Δ6 HER2 cells whereas EGF exposure increased the number of S-phase MCF10A/pLXSN and MCF10A/HER2 cells up to 13% and 15%, respectively. 86% of MCF10A/Δ6 HER2cells while only, 75% and 67% in MCF10A/pLXSN and MCF10A/HER2 cells, respectively accumulated in G_1-phase following treatment with EGF (Fig. 4B, bottom panels). Δ6 HER2 inhibits HER2-induced *in vitro* transformation and chemoresistant phenotype in MCF10A cells.

Colony formation assays in soft-agar clearly showed the ability of wild-type HER2 to confer anchorage-independency when overexpressed in MCF10A cells. MCF10A cells stably expressing Δ6 HER2 were unable to form colonies in soft-agar assays (Fig. 4C). We next tested the sensitivity of MCF10A/pLXSN, MCF10A/HER2 and MCF10A/Δ6 cells to the paclitaxel (PXT). HER2 overexpression promoted resistance to PXT, with an approximately 9-fold higher IC_{50} value (the concentration of the drug required to reduce by 50% cell viability) as compared to control cells (IC_{50} = 90 nM; 95% CI = 85 nM-95 nM and IC_{50} = 10 nM; 95% CI = 7 nM-13 nM, respectively; Fig. 4D). Overexpression of the Δ6 HER2 failed to promote PXT resistance (IC_{50} = 6 nM; 95% CI = 4 nM-8 nM). These findings strongly suggest that deletion of residues 451-466 is sufficient to abolish the ability of MCF10A/HER2 transformation and chemoresistance.

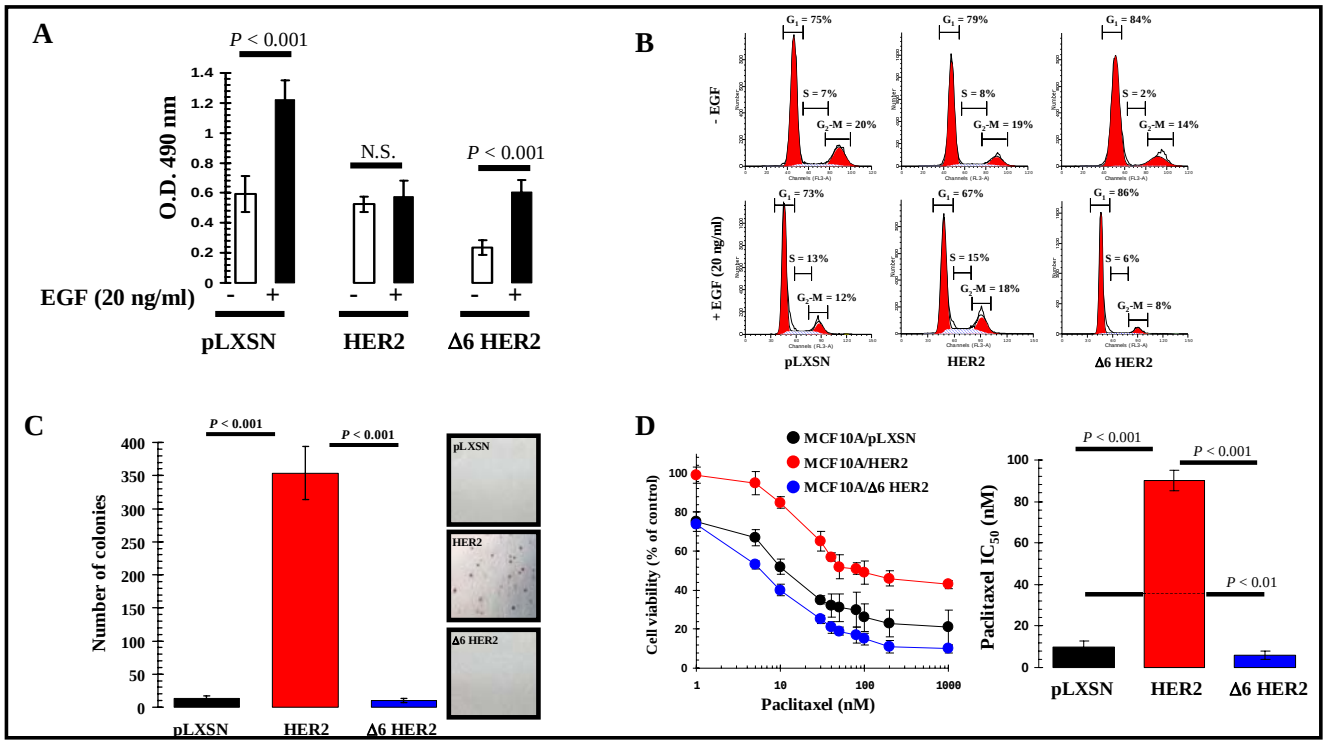

Figure 4: *Effects of Δ6 HER2 on the canonical oncogenic properties of HER2.* **A.** Effects of overexpressing either the wild-type form of HER2 or the structural mutant Δ6 on the EGF-dependency of MCF10A cells. MCF10A/HER2, MCF10A/Δ6 HER2 and MCF10/pLXSN cells were cultured in the presence of EGF. The levels of sensitivity to EGF ($P < 0.001$) compared with control (untreated) group). **B.** Effects of overexpressing either the wild-type form of HER2 or Δ6 HER2 on EGF-independent and EGF-stimulated cell cycle progression in MCF10A cells. After an overnight period of serum starvation and re-feeding with DMEM/F12-0.1% horse serum (HS) at time 0, Cells were treated with EGF for 24 h. **C.** Effects of overexpressing either the wild-type form of HER2 or Δ6 HER2 on the anchorage-independent phenotype of MCF10A cells. Cells (10,000 per well) were seeded in medium containing 0.35% low-melting agarose over a 0.7% agarose base layer and incubated for 14 days in DMEM/F12 supplemented with 10% HS and 20ng/ml EGF. ($P < 0.001$ for MCF10A/HER2 cells *versus* all other cell lines). All statistical tests were two-sided. **D.** Effects of overexpressing either the wild-type HER2 or Δ6 HER2 on chemotherapy-induced cell damage in MCF10A cells. Cells (2,000-3,000 per well) were cultured in graded concentrations of PXT. The cell viability effects from exposure of cells to PXT (*left panel*) were analyzed by generating concentration-effect curves as a plot of the fraction of unaffected (*surviving*) cells *versus* drug concentration. Each experimental value on the graph (*right panel*) represents the mean IC_{50} value (*columns*) and 95% confidence intervals (*bars*) from three separate experiments. IC_{50} values were designated for the concentrations of PXT (nM) absorbance values of cultured MCF10A-derived cells at 490 nm by 50% as determined using the MTT viability assay. One-factor ANOVA was used for all of the experiments.

5. PM2 blocks HER2 autophosphorylation and HRG-induced phosphorylation of HER-receptors: To characterize the PM2 effects, we analyzed the ability of PM2 to prevent phosphorylation of HER2 receptor induced by HRG. We used *in vivo* and *in vitro* tyrosine phosphorylation assays in cell culture in 10% serum to assess whether we can do indeed see a "REAL" inhibitory effect by the peptide. HRG and the peptide were pre-incubated prior to the phosphorylation assays. In MDA-MB-453 cells (+ serum), which that express the HER2 receptor. The peptidomimetic inhibited the baseline and HRG-stimulated levels of HER2 phosphorylation by 70-96% (Fig. 5). *Results revealed that PM2 caused a marked decrease in the phosphorylation of tyrosine residues, both in the absence (50% inhibition) and presence of HRG, 50% and 36% inhibition, respectively.*

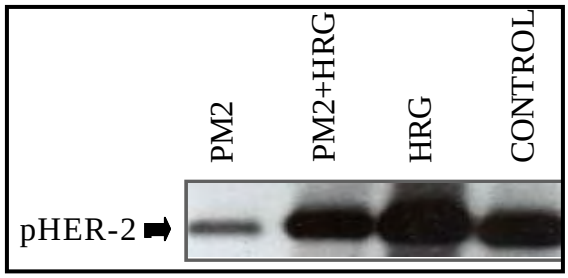

Figure 5: *Effect of PM2 on HER2 in vivo Phosphorylation:* Cells were grown to 80o/5 confluence in a 35-mm dish (Costar). Fetal calf serum (FCS) was removed 16 hr prior to labeling. Cells were rinsed with phosphate-free DMEM and then incubated for 3 hr at 37°C with 1.0 mCi (1 Ci = 37 GBq) of ^{32}Pi per ml per dish ([^{32}P] orthophosphate). After 3 hr, cells were treated for 20 min at 37°C with samples (60μM PM2 in the presence or absence of 30 ng HRG). Cells were quenched on ice, and lysed. After pre-incubation with 10 μ1 of normal mouse IgG, the nonspecific complexes were clarified using protein A-Sepharose. The supernatant was incubated with a monoclonal anti-phosphotyrosine antibody and specifically eluted using 1 mM phenyl phosphate. A second immunoprecipitation was then performed using a polyclonal antibody against the erbB-2 C-terminal sequence. After precipitation with protein A Sepharose the pellets were washed with buffer and the pellet was then resuspended and loaded onto a 7.5% SDS/PAGE.

<u>6. PM2 inhibits anchorage-independent growth of MDA-MB-453 cells induced by a feeder layer of fetal fibroblasts that express HRG:</u> To determine the *in vitro* growth effects of PM2 in an *in vivo* microenvironment (epithelial/stromal interaction), cross-feeding experiments were performed in the presence and absence of the blocking peptides. HRG expressing (F+) or HRG non-expressing (F-) fetal fibroblasts were plated at different densities on a 35mm dish in monolayer (MDA-MB-453 cells in a soft- agar suspension were mixed with vehicle, PM1 (a control, non-specific peptide) or PM2 (50 μg/ml). The cell- suspension layer, including the treatments, placed on top of the bottom layer. The HRG-expressing fibroblasts induced the proliferation of MDA-MB-453 cells in a density-dependent manner, which was abrogated by PM2. PM1 displayed no effect.

Although, these experiments were not with the epithelial and stromal cells from the same patients, the purpose of the experiments was to demonstrate that the paracrine action of HRG and perhaps other growth factors that lead to HER2 receptor dimerization could be affected utilizing the peptido-mimetic agent generated against the Δ6 site. This is very important since most growth factors which induce cross-talk among the HER receptor family are produced in many cases by the stroma exclusively making the paracrine loop, and in other cases are produced by the epithelial cells making the autocrine loop the functional loop. Since we demonstrated with the mutants the "autocrine loop" relevance of the Δ6 site, it was pertinent to demonstrate that the "paracrine loop" the disruption of domains I and III interaction will be relevant as shown in Fig. 6.

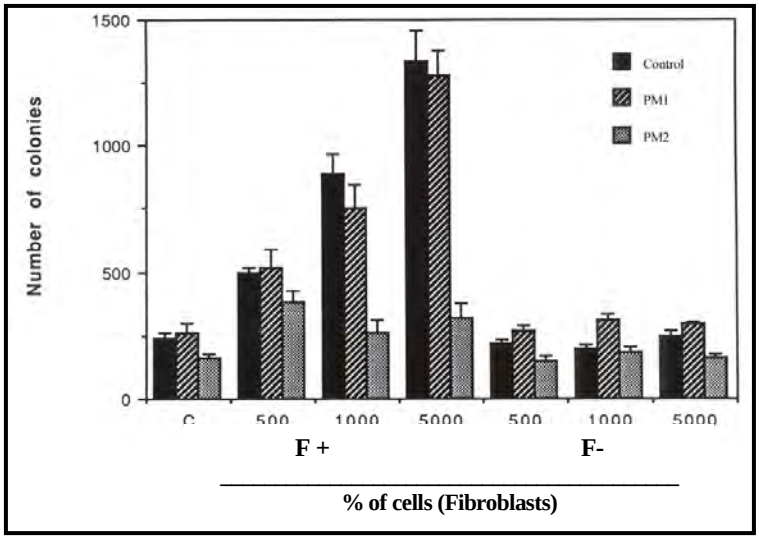

Figure 6: *Effect of PM2 on the growth of HER2 overexpressing cells over a feeder layer of fibroblast* PM2 specifically blocks the growth of HRG induced anchorage independent growth of MDA-MB-453 cells. HER2 overexpressing cells, MDA-MB-453, were plated on a 35 mm tissue culture dishes onto feeder layers of fetal fibroblast that do express HRG **(**F-), and do not express HRG (F+) at different cell densities. PM1 and PM2 were added to the top layer containing the cells, 0.4 % agar and 10 % FCS. Plates were incubated 7-9 days and colonies were counted.

7. Development of a Peptide synthesis: The following modifications have been performed to the leading compound in order to identify the best peptide and the essential amino acids that are important for the activity. To illustrate which amino acids are important in the lead structure for the observed activity an Alanine Scan was performed. We performed Alanine scan to demonstrate which of the amino acids in a peptide sequence were central for its activity. This was achieved by synthesizing several peptides performing a sequential single amino acid changes and substituted by Alanine. An increase or decrease in peptide activity was noted. The activity of each of the PM2-congers (PM2-CS) was determined in SK-Br3 cells. HER2 overexpressing cells were treated with increasing concentrations of the peptide PM2-CS to assess HER2 autophosphorylation, anchorage-independent growth and HER2 receptor localization by immunofluorescence. We determine which amino acids are crucial to the PM2 peptide activity and concluded that the last four amino acids of the PM2 peptide are essential for its activity as shown in Table 1.

Table 1: Activity of the Alanine modifications of PM2

PM2-Conger	Inhibition of HER2 activation	Modulation of HER2 localization	Inhibition of anchorage-independent growth
Unrelated Peptide	-	Membrane Staining	**No inhibition**
LGLRSLRE (PM2)	+++	Cytoplasmic and Nuclear Staining	**60%**
LGLRSLRA	-	Membrane Staining	**No inhibition**
LGLRSLAE	-	Membrane Staining	**No inhibition**
LGLRSARE	-	Membrane Staining	**No inhibition**
LGLRALRE	+/-	Membrane and Cytoplasmic Staining	**20%**
LGLA SLRE	++	Mostly cytoplasmic Staining	**35%**
LGAR SLRE	++++	Mostly Cytoplasmic and Nuclear Staining	**72%**
LALR SLRE	++++	**Cytoplasmic and Nuclear Staining**	**83%**
AGLR SLRE	++++	**Cytoplasmic and Nuclear Staining**	**87%**

The determination that the last four amino acids are essential for PM2- activity was demonstrated as sequential changes of each one of the last amino acids of PM2 with Alanine disrupted the activity of PM2, implying that to maintain that the core peptide is indeed in the last four amino acids.

This information is essential to the continuation of our studies since we have to maintain the "core peptide". To do so, we may need to either modify other sites of the peptide or just use these four amino acids as the "core peptide" and begin from this structure since these are necessary and sufficient to confer activity. We further will determine whether truncation of the PM2 peptide will modify the activity of this peptide we performed nested single amino acid truncations of the lead sequence.

KEY RESEARCH ACCOMPLISHMENTS

The increasing number of newly discovered anti-cancer agents is a result of better understanding the mechanisms underlying malignant transformation and metastatic potential rather than the random screening of molecules. The human epidermal growth factor receptor 2 (HER2 or *erb*B2), a transmembrane tyrosine kinase protein, represents a successful example of this molecular approach. Experimental studies demonstrating that high levels of HER2 transform cultured cells and clinical studies showing that patients whose tumors overexpress HER2 have poorer long-term survival rates implied that HER2 is a suitable therapeutic target. While various approaches have been taken to target this molecule, the most prominent strategy for the treatment of HER2-overexpressing carcinomas has involved antibody-targeting the extracellular domain (ECD) of HER2, such as trastuzumab and pertuzumab. Unfortunately, targeting HER2 is not as straightforward as it was originally predicted since many breast tumors express multiple HER receptors and co-express one or more HER ligands. This ***cross-talk network*** negatively impacts the response to the currently used HER2-targeted agents, highlighting the urgent need for a novel anti-HER2 molecule(s) presenting a ***combination strategy***. Activation of the HER2 receptor is attained by several means: 1) receptor overexpression leads to homodimerization; 2) EGF induces EGFR-HER2 hetero-dimerization by binding to EGFR and inducing HER2 activation via crosstalk; and 3) Heregulin (HRG) induces HER3-HER2 hetero-dimerization by activating HER2 via its binding to the HER3 and HER4 receptors, in turn inducing receptor crosstalk and activation of a signaling cascade. In short, our preliminary study reveals that the specific disruption of an essential activating sequence existing on HER2 ECD domain III is capable of disabling the HER2 homo- and hetero-dimerization, thus blocking activation of HER2-driven oncogenic signaling and generating a “dominant-negative” (and likely autoinhibited) form of HER2. Peptides or compounds with specificity for this functional site should add a previously unrecognized molecular approach to our therapeutic arsenal for the management of HER2-overexpressing carcinomas.

Our data reveals that specific disruption of an essential activating sequence existing on HER2 ECD domain III is capable to disable the HER2 homo- and hetero-dimerization loop. Thus, blocking subsequent activation of the HER2-driven oncogenic signaling and further generating a “dominant-negative” -likely auto-inhibited- form of HER2, offers a strong rationale to develop this peptide sequence into a valuable anti-HER2 therapeutic drug. Monoclonal antibodies with specificity for this functional site should add a previously unrecognized molecular approach broaden our therapeutic arsenal for the targeted management of HER2-overexpressing carcinomas

REPORTABLE OUTCOMES

Here we present evidence that, indeed, specific disruption of a small core sequence within domain III of HER2 ECD is sufficient to disable HER2, blocks downstream activation of HER2-driven oncogenic signaling and further generates a “dominant-negative” form of HER2. These findings suggest a new avenue for interfering therapeutically with HER2 activation by directly targeting its autoactivation mechanism. Our results with Δ6 HER2imply that deleting a small portion of the subdomain III from the HER2 ECD disrupts interactions that are normally responsible for stabilizing the constitutive dimerization-competent (extended) conformation of HER2. Although future structural studies should clarify this assumption, it is obvious that disruption of this “activating sequence” in the ECD of HER2 should prevent the constitutive juxtaposition of domains I and III when considering that:

- Δ6 HER2 failed to promote any basal HER2 dimerization and HER2 *auto*-phosphorylation, while notably preventing *trans*-phosphorylation of HER2 by ligand-bound HER1 or HER3, when overexpressed in HER2-negative MCF10A cells and

- Δ6 HER2 significantly decreased constitutive overactivation of HER2 and markedly reduced HER2-catalyzed *trans*-activation of HER1 and HER3 when overexpressed in breast cancer cells naturally or ectopically overexpressing the wild-type form of HER2 (SK-Br3 and MCF-7/HER2-18 cells, respectively).

These findings reveal for the first time that an essential activating sequence exists on the domain III of the HER2 ECD as its disruption disables the HER2 homo- and hetero-dimerization loop, blocks subsequent activation of the HER2-driven oncogenic signaling, and generates a dominant-negative form of HER2. High affinity peptides and/or antibodies with specificity for this molecular-activation switch may represent a novel targeted approach for the management of HER2-overexpressing carcinomas. Thus, we generated a peptidomimetic compound PM2 and determined that PM2 is a leading peptidomimetic agent capable of blocking tumor growth of HER2 overexpressing breast cancer cells.

CONCLUSION

Our data reveals that specific disruption of an essential activating sequence existing on HER2 ECD domain III is capable to disable the HER2 homo- and hetero-dimerization loop, thus blocking subsequent activation of the HER2-driven oncogenic signaling and further generating a “dominant-negative” -likely auto-inhibited- form of HER2, offers a strong rationale to develop this peptide sequence into a valuable anti-HER2 therapeutic drug. Monoclonal antibodies with specificity for this functional site should add a previously unrecognized molecular approach broaden our therapeutic arsenal for the targeted management of HER2-overexpressing carcinomas

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APPENDICES

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